METHOD 4425

SCREENING EXTRACTS OF ENVIRONMENTAL SAMPLES FOR PLANAR ORGANIC COMPOUNDS (PAHs, PCBs, PCDDs/PCDFs) BY A REPORTER GENE ON A HUMAN CELL LINE

1.0 SCOPE AND APPLICATION

1.1 Method 4425 utilizes a reporter gene system (RGS) based on cytochrome P450 to screen samples for a range of organic compounds including polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), coplanar polychlorinated biphenyl congeners (PCBs), and high molecular weight polynuclear aromatic hydrocarbons (PAHs).

1.2 The method is a screening procedure that will detect the total amount of planar compounds in solvent extracts of environmental samples of soil, sediment, tissue, and water. Since the concentration of solvent will be less than 1% in the medium containing the cells (2 to 20 µL in 2 mL), the choice of solvent is not critical. The solvents already tested successfully are DMSO, methylene chloride, hexane, methanol, isooctane, and acetone.

1.3 This screening procedure will correctly identify samples containing these planar compounds above the detection limits in Table 1 at least 95% of the time.

1.4 The test results are a function of the concentrations and potencies of specific CYP1A1-inducing compounds within the mixture extracted from the environmental sample. The results integrate the cell responses induced by the compounds that are present in the sample, and have been shown to be approximately additive. The initial measurement of cellular response in the solvent blank is divided into that of the standards and the sample extracts to determine the "fold induction," or "times background."

1.5 The detection limits for specific PAHs, PCBs, PCDDs, and PCDFs are shown in Table 1. Detection limits can be adjusted to meet the needs of a project by altering the weight of the sample and the extent of extract concentration. If smaller samples are used, the detection limits will increase, unless the volume of solvent is reduced below 1 mL. The sensitivity of the test will be influenced by the specific composition of the contaminants at a site. The test should produce internally-consistent results at any given site.

1.6 A tiered testing approach is recommended.

1.6.1 Tier I is the testing of many sample extracts at 16 hours of exposure to determine the range of responses from a site.

1.6.2 Sample extracts representing the full range should then be tested in Tier II at 6 and 16 hours to observe the differences. Since PAHs can be degraded by the enzymes in the cells, they produce peak induction at 6 hours, while the induction due to dioxins and coplanar PCBs will increase from 6 to 16 hours of exposure.

1.6.3 Tier III is the quantitative chemical characterization of samples representing the full range of RGS responses and utilizing traditional instrumental analytical methods, and the relationship between the responses after 6 hours and 16 hours may be used to select the target analytes for the instrumental analyses.

1.6.4 In Tier IV, the RGS responses are correlated with the concentrations determined by traditional quantitative instrumental methods (e.g., GC/MS). If there is a good correlation (r^2 = >0.6), concentrations of the remaining samples may be estimated from the curve and slope. If appropriate for the intended application, these findings may be used to determine the extent of contamination or the rate of remediation.

1.7 In cases where exact concentrations of specific PAHs, PCBs, or PCDDs/PCDFs are required, quantitative techniques should be used. Results from screening with this method should provide guidance on the selection of the most appropriate samples for chemical analyses.

1.8 Because the procedure involves testing of concentrated solvent extracts of field samples and a 6- to 16-hour incubation of the cell solution under controlled conditions, it is not a field screening procedure. The method is most likely to be employed in a fixed laboratory, although it may be possible to conduct such screening in a well-equipped mobile laboratory or on-site facility.

1.9 Prior to employing this method, analysts are advised to consult the base method for each type of procedure that may be employed in the overall analysis (e.g., Methods 3500 and 4000) for additional information on quality control procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts also should consult the disclaimer statement at the front of the manual and the information in Chapter Two, Sec. 2.1, for guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies, and on the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrix of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly specified in a regulation, the use of SW-846 methods is *not* mandatory in response to Federal testing requirements. The information contained in this method is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to generate results that meet the data quality objectives for the intended application.

1.10 This method is restricted to use by or under the supervision of trained analysts familiar with cell culture techniques. Each analyst must demonstrate the ability to generate acceptable results with this method. Training opportunities may be available from the method developer (see Sec. 6.2.1 for contact information).

2.0 SUMMARY OF METHOD

2.1 The reporter gene system utilizes a human cell line (101L) into which a plasmid containing a human CYP1A1 promoter and 5'-flanking sequences fused to a reporter gene,

firefly luciferase, have been stably integrated. In the presence of CYP1A1-inducing compounds, the enzyme luciferase is produced, and its reaction with luciferin can be detected by measuring relative light units (RLUs) in a luminometer. CYP1A1-inducing environmental contaminants include polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), coplanar polychlorinated biphenyl congeners (PCBs), and high molecular weight polynuclear aromatic hydrocarbons (PAHs).

2.2 Soil, sediment, tissue, and water samples to be tested are extracted by appropriate methods and solvents. Extraction should not include the addition of surrogates or internal standards unless previous testing has shown that the assay does not respond to these compounds. The solvent volume is reduced to 1 mL and an aliquot of 2 to 20 µL of the sample extract is applied to each of three wells in a tissue culture plate. Each well contains cells from the 101L line in 2 mL of culture medium. In general, the lower volume (2 µL) is used when sample extracts exhibit a very dark (brown or black) color, or when the samples are known to be highly contaminated. The same solvent and volume are used for the three replicates of the solvent blank. Solvents tested successfully are methylene chloride, DMSO, hexane, methanol, isooctane, and acetone.

2.3 To quantify the inducing compounds in the sample, the mean response, in RLUs, of the three sample replicates is divided by the mean response of three replicates of a solvent blank, yielding a "fold induction," which is a measure of the increase of the sample response over the background response. Fold induction may be converted to toxic equivalents (TEQ) for PCBs and PCDDs/PCDFs, or benzo[a]pyrene equivalents (B[a]PEq) for PAHs, based on the fold induction responses to standards containing a mixture of PCDDs/PCDFs, or benzo[a]pyrene, respectively.

2.4 This method can be used to estimate the concentrations of PAHs and/or coplanar PCBs in soils over a range from 0.1 to over 100 mg/kg. In addition, the presence of PCDDs/PCDFs in the extract will be indicated at concentrations ranging from 100 ng/kg to over 1 mg/kg. These sensitivity estimates are based on the extraction of 40-g solid samples and evaporation of the extract to 1 mL, with the application of 20 µL to one million cells covered by 2 mL of medium. The sensitivity of the method for water samples is approximately 25 times greater, assuming that a 1-L water sample is extracted and the extract is concentrated to the same 1-mL volume (e.g., 4 µg/L to 4 mg/L for PAHs and PCBs, and 4 ng/L to 40 µg/L for PCDDs/PCDFs).

2.5 The method contains an optional procedure in which exposures are conducted over two specific time periods (6 and 16 hours). This option allows the test to distinguish between PAHs in the samples and chlorinated compounds, since the PAHs reach maximum induction at 6 hours, while the peak in induction from chlorinated planar compounds (PCBs, PCDDs/PCDFs) is not until 16 hours.

2.6 This method is a screening procedure, and depending on project needs, a subset of the samples should be confirmed using quantitative analytical techniques.

3. 0 DEFINITIONS

Definitions associated with immunoassay procedures are given in Method 4000 and many apply to this procedure. Additional definitions are provided in the glossary at the end of this method.

4.0 INTERFERENCES

4.1 The chemicals listed in Table 2 have been tested and found not to interfere with the response of the RGS assay to the compounds listed in Table 1. As yet, there has been no evidence that any pesticide has the appropriate molecular structure to interfere with the response of this test system. The assay has identified approximate concentrations of PCDDs/PCDFs at µg/kg levels in environmental samples in the presence of 100 mg/kg levels of pentachlorophenol. Metals will not interfere, since they will not likely be present in the solvent extracts. A concentration of 10 mg/kg or greater of tributyl-tin (TBT) will inhibit this test system and produce cell death, but this level of TBT contamination is very unlikely. Significant mortality of the cells would be observed by the analyst.

4.2 This test does not respond to 1- to 3-ring PAHs, but does respond to 4- to 6-ring PAHs to varying degrees (see Table 1). It is likely to respond to alkylated forms of these higher molecular weight PAHs, but none have been tested thus far. The co-occurrence of PAHs, coplanar PCBs and PCDDs/PCDFs will produce a response that is near additive. It is not possible with this assay to separate the response from coplanar PCBs from that of PCDDs/PCDFs. As these two groups of chlorinated compounds are both ranked for potential risk on a basis of toxic equivalents (TEQs), it is appropriate that the result of a test is expressed as a TEQ.

4.3 The extracts of some soil samples that were extremely high in either PAHs (over 1,000 mg/kg) or chlorinated compounds (several hundred mg/kg pentachlorophenol) have produced toxicity for the cells. The first method of determining if such toxicity has occurred is to observe floating cells, which have released from their healthy condition of attachment to the bottom of the well. A second check of toxicity is the relative standard deviation (RSD) of the replicates, since this will increase above the acceptance level of 20%. When a sample or group of samples is suspected of being highly contaminated with PAHs, it is advisable to test dilutions of the extract, in addition to the full strength extract. An example of an appropriate dilution series is full strength, 1:10 and 1:50. If fold induction increases from one dilution to the next, then toxicity has produced the lower response at the higher concentration.

4.4 Because the test responds to PAHs, PCBs, and PCDDs/PCDFs, any one of these groups of compounds could be considered an interference with the analysis of the other group(s). Therefore, this method includes an optional procedure that will discriminate between the response to PAHs and chlorinated hydrocarbons in a sample by conducting testing on replicates of an extract for both 6 hours and 16 hours. This option may be selected initially, or it may be used in follow-up testing with sample extracts that produced high responses (high contamination) in the 16 hour test. See Sec. 11.7 for information on the optional procedure.

5.0 SAFETY

5.1 Safety procedures consistent with good laboratory practices should be used. Some reagents may contain dilute acids, and solvent extracts of samples may contain hazardous materials. The analyst should always avoid contact of eyes, skin, and mucous membranes with these solutions. Waste solutions should be placed in the appropriate safety container and disposed of in accordance with all applicable state and federal regulations.

5.2 This method employs dilute standards containing 2,3,7,8-TCDD for calibration. The analyst should take appropriate precautions when preparing, handling, and disposing of these standards.

6.0 EQUIPMENT AND SUPPLIES

6.1 Equipment

6.1.1 Microcentrifuge - capable of holding 6,000 rpm for 10 seconds, and holding 24 1.5-mL tubes. Fisher model 16KM, #04-977-16KM, or equivalent.

6.1.2 Microcentrifuge tubes - 1.5-mL, plastic. VWR #20901-551 or equivalent.

6.1.3 Dynatech luminometer ML2250, or equivalent, capable of detecting 2 pg of luciferase or less.

6.1.4 Vertical laminar flow hood - VWR #21917-126 or equivalent.

6.1.5 CO_2 incubator - water-jacketed, VWR #35909-751, or equivalent.

6.1.6 Heated water bath - 5.5-L, VWR #13470-030, or equivalent.

6.1.7 Poppette positive displacement micropipettor - 0.5-mL to 15-mL, Fisher #21-117-3, or equivalent.

6.1.8 Oxford Benchmate 200-mL pipette - VWR #40000-204 or equivalent.

6.1.9 Oxford Benchmate 1000-mL pipette - VWR #40000-208 or equivalent.

6.1.10 Drummond portable pipet aid - VWR #53498-103 or equivalent.

6.1.11 Refrigerator-freezer - capable of maintaining 4°C in the refrigerator and at least -20°C in the freezer.

6.1.12 Liquid nitrogen Dewar flask - 10-L capacity, VWR #55708-604, or equivalent.

6.1.13 Top loading balance - 0 - 50-g range, readability 0.1 mg, repeatability 0.1 mg, Fisher #01-913-447, or equivalent.

6.1.14 Centrifuge - capable of holding 8 50-mL tubes and rotating at 1,000 rpm for 5 minutes, VWR #20671-012, or equivalent.

6.1.15 Freezing rate controller - Gordinier Electronics Model 7009, or equivalent.

6.2 Supplies

6.2.1 The 101L cell culture, available from Columbia Analytical Services, 1185 Park Center Drive, Suite A, Vista, CA 92083 (www.caslab.com).

6.2.2 Cell scraper, sterile (individually wrapped), plastic, disposable - Fisher #08-773-2, or equivalent.

6.2.3 Poppette micropipettor tips and plungers - Fisher #21-117-10 or equivalent.

6.2.4 Oxford Benchmate 1-200 mL pipette tips - Fisher #21-197-8H or equivalent.

6.2.5 Oxford Benchmate 200-1000 mL pipette tips - Fisher #21-197-8J or equivalent.

6.2.6 6-well tissue culture plates - Fisher #08-772-1B or equivalent.

6.2.7 Vented tissue culture flasks - Fisher #10-126-12 or equivalent.

6.2.8 Disposable plastic pipettes - 2-mL, 10-mL, and 50-mL. Sterile, individually wrapped.

6.2.9 Plastic centrifuge tubes - 15-mL and 50-mL, sterile.

6.2.10 Cryogenic vials, plastic, 2-mL, sterile - Fisher #03-374-6.

6.2.11 Sterile filters, 0.22-mm pore size, 25-mm - Fisher #09-740-480 or equivalent.

6.2.12 Sterile, plastic, disposable, syringes, 10-mL - Aldrich #Z24-803-7 or equivalent.

7.0 REAGENTS AND STANDARDS

7.1 Methylene chloride, CH_2Cl_2, HPLC grade.

7.2 Dimethyl sulfoxide (DMSO), $(CH_3)_2SO$, molecular biology grade.

7.3 2,3,7,8-TCDD - 1 mL of a solution of 10 µg/mL in toluene, Ultra Scientific #RPE-029S, North Kingstown, RI, or equivalent.

7.4 Luciferase standard curve kit - PharMingen, San Diego, CA, or equivalent.

7.5 Substrate A - Commercial buffer containing cofactors (ATP, Mg, etc.) necessary for the luciferase reaction. PharMingen, San Diego, CA, AL #1801-50, or equivalent.

7.6 Substrate B - Commercial buffer containing luciferin, the substrate of the luciferase reaction. PharMingen, San Diego, CA, AL #1802-50, or equivalent.

7.7 Cell lysis buffer - PharMingen, San Diego, CA, AL #1820, or equivalent.

7.8 Eagle's minimal essential medium - Fisher #MT-15-010-LV or equivalent.

7.9 Hank's balanced salt solution - Fisher #MT-21-021-LV or equivalent.

7.10 L-glutamine - Sigma #G6392 or equivalent.

7.11 Geneticin-G418 - Sigma #G9516 or equivalent.

7.12 Trypsin - Sigma #T4424 or equivalent.

7.13 Sodium pyruvate - Sigma #S8636 or equivalent.

7.14 Fetal bovine serum (FBS) - Sigma #F3018 or equivalent.

7.15 Penicillin/Streptomycin - Fisher #30-002-LI or equivalent.

7.16 Sodium sulfate - Fisher #S415-500 or equivalent.

7.17 Organic-free reagent water - All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 See Chapter Four, Organic Analytes, Sec. 4.1, for sample collection and preservation instructions.

8.2 Tissue samples can be frozen and held in a freezer in preparation for shipping.

8.3 Samples may be extracted and tested at the same laboratory, or the extracts may be prepared in one laboratory and shipped to another laboratory for RGS testing. When sample extracts are shipped, they should be concentrated to a volume of approximately 1 mL and sealed in either crimp-sealed vials or vials with PTFE-lined screw caps.

8.4 Extracts should be stored either frozen, or under refrigeration in the dark.

8.5 No holding times have been established in conjunction with the RGS screening method. However, the target compounds (dioxins, furans, PCBs, and PAHs) are extremely stable in environmental matrices.

9.0 QUALITY CONTROL

9.1 Initial calibration

A single-point initial calibration of the response of the RGS assay to dioxin (TCDD) must be performed concurrent with the analysis of any samples, as described in Secs. 10.1 and 11.6. Unless other concentrations are needed for a specific project, the assay is calibrated with a 1 ng/mL standard of 2,3,7,8-TCDD. Historical data indicate that 1 ng/mL of TCDD should produce a mean test response of approximately 110 fold induction, with a standard deviation of 21 (see Figure 1).

9.2 Calibration verification

Calibration verification is not performed in the traditional sense, because the initial calibration standard is analyzed with each batch of samples each time the analyses are performed.

9.3 Routine quality control procedures

Routine quality control procedures associated with this method include the analyses of three replicates of all standards, sample extracts, and solvent blanks. Other quality control samples such as matrix spike samples and laboratory control samples (LCS) may be included in an analytical batch. However, since this method is a screening procedure, these additional measures are not needed for most applications.

It is critical that the laboratory maintain quality control charts (see Figure 1) showing a mean for the response of the method (fold induction = times the solvent blank) to 1 ng/mL of TCDD (or lower concentration) for each day on which samples are analyzed. If the test response is outside of two standard deviations from this mean, the samples in the affected batch must be retested. If the relative standard deviation (RSD) for the three replicates of a sample is greater than 20%, that sample should be retested.

If an LCS spiked with a PCDD/PCDF mixture is analyzed with a batch of samples, the response should be compared to a concentration-response curve (see Figure 2).

9.4 Sample dilutions

If the sample produces a response of greater than 100-fold induction, then the sample should be diluted with the same solvent and retested. Samples known to span a wide range of concentrations can be initially tested at full strength, and two dilutions (e.g. 1:10 and 1:50), to avoid the need for subsequent dilution and retesting.

9.5 Other quality control considerations

9.5.1 Standards, reagents, and particularly solutions used in measuring luminescence, must be stored properly and must not be used past the expiration dates provided by the suppliers.

9.5.2 The laboratory must demonstrate the sensitivity and linearity of the luminometer, at least monthly, with a luciferase standard. At a minimum, two replicates each of 2, 10, 50 and 100 pg/mL of luciferase should be tested. The instrument should detect 2 pg of luciferase and the standard curve should be linear, with an r^2 value of at least 0.9 (see Figure 3).

10.0 CALIBRATION AND STANDARDIZATION

Calibration is performed concurrently with the analysis of a batch of samples. Three replicates of a 1.0 ng/mL standard of TCDD should be analyzed with each batch of samples. (Other TCDD concentrations or other CYP1A1-inducing compounds may be used, as appropriate for the specific project). The mean fold induction measured for these replicates should be compared to the running mean of the control charts to be certain that the mean response is within two standard deviations of the overall mean. If the mean fold induction of the calibration standard is outside of the appropriate range, then the testing of the affected batch must be repeated.

11.0 PROCEDURE

This section describes the procedures to maintain the human cell line in culture, to prepare the cells for use in the test, to conduct the test itself, and an optional procedure to differentiate between the responses of PAHs and the chlorinated target compounds (PCDDS/PCDFs and PCBs).

11.1 Cell culture maintenance

The transgenic 101L cell line requires incubation at 37°C in an atmosphere of 5% CO_2 and 100% humidity. The maintenance of the cell line requires the use of plastic tissue culture flasks (to which the cells adhere) and changes of media every 3 to 4 days. To prepare media, add the following reagents to a 500-mL bottle of Eagle's Minimum Essential Media (without L-Glutamine):

50 mL	Fetal bovine serum (10%)
10 mL	200 mM L-Glutamine (2%)
5 mL	Sodium pyruvate (1%)

With each change of media, Geneticin (antibiotic G418) at a final concentration of 0.4 g/L must be added to control for the resistant 101L cells and eliminate cells not containing the plasmid. G418 is prepared at a concentration of 40 g/L by adding 25 mL sterile reagent water (filtered through 0.2-µm filter) to 1 g of Geneticin disulfate salt. This is a convenient

concentration for a 1:100 dilution (e.g. 150 µL of G418 solution is added to 15 mL of media in flask).

Media should be stored at 4°C. All other reagents may be divided into aliquots and frozen at -20 °C. When adding media to cells, warm the media to 37°C in a water bath.

NOTE: ALWAYS use sterile techniques when working with cells.

11.2 Thawing cells

The 101L cell line is stored in liquid nitrogen in 2-mL cryogenic vials. In order to establish a new culture of viable cells, the frozen cells must first be carefully thawed. Warm the cell media to 37°C in a water bath before thawing the cells. Transfer the cryogenic vial to be thawed into a small container of liquid nitrogen to keep cells frozen until immediately before thawing. Thaw the cells by plunging the vial into a 37 °C water bath. As soon as the ice has melted, GENTLY transfer the cells into a sterile 15-mL centrifuge tube. SLOWLY drip 12 - 15 mL of warm media (without G418) into the tube containing the cells and transfer the solution into a tissue culture flask. Once the cells have adhered to the flask (4 - 5 hours), pipette off the media and add fresh media that contains G418.

11.3 Splitting and counting cells

As the cells in the culture mature, maintaining the culture requires that the cells must occasionally be split into smaller aliquots of approximately the same number of cells. When the cells in a flask become confluent, trypsinize 1 - 2 times/week to divide into a new flask or 6-well culture plates. Prepare the growth media and warm to 37°C in water bath. Thaw the trypsin. Under a sterile hood, pipette off the media from the flasks and add 5 mL of trypsin to each flask. Tighten the flask lid and place the flask horizontally in incubator, making sure that the trypsin covers entire surface of cells. Allow 5-10 minutes for the cells to dissociate from the surface, then place the flask under the hood and add 5-10 mL of growth media. Gently pipette the media down the surface of the flask to remove the cells. Place the cell solution into a sterile 50-mL centrifuge tube and spin at 1000 rpm for 5 minutes. Pour off as much of the media containing trypsin as possible and resuspend cells in 20 mL of fresh growth media, GENTLY pipetting up and down to break up clumps.

To count the cells, place 100 µL of cell solution into microcentrifuge tube along with 400 µL of Hank's salt solution (need not be sterile). Mix the cells and apply to a hemocytometer under a cover glass. Count both sides of the hemocytometer (each a 4 x16 grid) and take the average of two counts. Divide the average by 4, multiply by 5 (cell dilution in Hank's), multiply by 10,000 (hemocytometer factor), and then multiply by 20 (for 20 mL cell solution). This is the total number of cells in the 20 mL of cell solution.

For each 6-well plate, make up enough cell solution for 7 wells (1.75 million cells). For 6 plates, a total of 84 mL of cell solution is needed (containing 10.5 million cells). Add G418 solution so that the concentration in the cell solution is 0.4 g/L (add 840 µL to 84 mL of cell solution). Swirl to mix and add 2 mL to each well of the plates. Remaining cell solution should be added to a new flask at 1-2 million cells/flask.

11.4 Freezing cells

Maintain the frozen culture by freezing cells in the log phase of growth (1-2 weeks after thawing and 2-3 splits). The equipment necessary is liquid nitrogen and a freezing rate controller. Be sure to have a full supply of liquid nitrogen before starting the freezing procedure.

Prepare freeze media by the following recipe for 5 mL total:

3.3 mL	Cell culture media (with all ingredients except G418)
1.25 mL	Fetal bovine serum (25%)
0.4 mL	DMSO (8%)
0.05 mL	Penicillin/streptomycin (1%)

Keep the media on ice at 4 - 6°C. Remove any adherent cells from flasks with trypsin. Gently form a pellet by centrifugation at 1,000 rpm and resuspend the cells in the freeze medium at 10^6 - 10^7 cells/mL (one 80% confluent flask/mL). Aliquot the cells into freezing vials, keeping them on ice until freezing begins.

Place a cryovial containing 1 mL of freezing media (no cells) in the middle of the rack in the freezing apparatus. Put the temperature probe in the tube and attach the liquid nitrogen dispenser to the apparatus. Start the freezing chamber ("prep chamber mode") and run until the temperature of the chamber and the freezing media are within 4°C of each other. Place the cryovials containing the cell solution in the chamber and turn the mode switch to "run program." The chamber will slowly freeze the cells at an appropriate cooling rate. The process takes approximately one hour, at which point vials can be placed into long-term storage in liquid nitrogen.

NOTE: If freezing equipment from other manufacturers is employed, follow manufacturer's instructions regarding the specific settings and operation.

11.5 Preparation for testing

11.5.1 Determine the total number of samples, solvent blanks, and standards to be used in one batch. Multiply that number by three replicates to determine the number of wells to be prepared. Transfer 0.25 x 10^6 cells to each well in a 6-well plate in 2 mL of media (1.5 x 10^6 cells per plate) until enough wells have been prepared for all the replicates.

11.5.2 Place the plates in an incubator at 37°C and 5% CO_2 for 3 days, to provide growth in numbers of cells and assure adhesion to the plastic. The 2 mL of media in each well will not be changed before the addition of the sample extract (see Sec. 11.6).

11.6 Conducting the test

11.6.1 Prepare a solution of 2,3,7,8-TCDD at a concentration of 1.0 ng/µL. Other TCDD concentrations or other CYP1A1-inducing compounds may be used, as appropriate for the specific project.

11.6.2 Prepare the solvent blank from the same solvent used for extracting the samples to be tested.

11.6.3 Bring the sample extracts, reagents, and standards to room temperature (approximately 20°C), with the aid of a water bath to speed the process.

11.6.4 With a positive displacement (Poppette) micropipettor, apply 2 µL of a 1 ng/µL solution of TCDD to 3 wells (2-mL well volume) for a final concentration of 1 ng/mL. (Other TCDD concentrations or other CYP1A1-inducing compounds may be used, as appropriate for the specific project).

11.6.5 Apply the solvent control to three wells at the same volume as the test substances (2 - 20 µL).

11.6.6 Apply each sample extract to three replicate wells. The volume applied depends on the solvent. Any of the solvents listed in Sec. 1.2 may be applied in volumes up to 10 µL, and DMSO and methanol may be applied up to 20 µL.

11.6.7 Incubate the plates for 16 hours in an incubator at 37°C and 5% CO_2.

11.6.8 After 16 hours, aspirate the media and rinse the cells with a saline solution (Hank's balanced salt solution) by applying the solution slowly to the side of the well with a 10-mL pipette. Be sure to dispose of media containing TCDD or other inducers in a proper waste disposal container.

11.6.9 Add 200 µL of the lysis buffer to each well with a pipette (Oxford 1-200 µL, or equivalent) and incubate the plate for 5 - 15 minutes at 4°C.

11.6.10 After this incubation, scrape the contents of each well and transfer approximately 200 µL of the suspension to a microcentrifuge tube using a pipette (Oxford 1-200 µL, or equivalent).

11.6.11 Spin the cells for 10 seconds at 6,000 rpm to separate the cellular debris.

11.6.12 Using a pipette (Oxford 1-200 µL, or equivalent), add 50 µL of the supernatant of each sample to a separate well of a 96-well luminometer plate. Duplicate analyses may be conducted if desired, with an additional 50-µL sample of the supernatant.

11.6.13 The luminometer dispenser should be stored in reagent water. Flush out the reagent water from the dispenser and prime it with Substrate B (luciferin), making sure to remove all air bubbles from injection tubes.

11.6.14 Use a pipette to add 100 µL of Substrate A to each well and place the plate into the luminometer. Set the luminometer on the integrate mode, at medium gain, so that 100 µL of Substrate B is automatically injected by the luminometer dispenser into each well and relative light units are measured at regular intervals. A pipette may be used to manually add Substrate B, but it must be added within 10 minutes of Substrate A, and the luminometer reading should be taken less than 5 minutes after adding Substrate B.

11.6.15 Record the relative light units (RLUs) for each well (sample) from the luminometer.

11.7 Optional procedure to discriminate PAHs from PCDDs/PCDFs and PCBs

The test can be used to discriminate between the responses to PAHs and the chlorinated compounds (e.g, PCDDs/PCDFs and PCBs) in a sample, by incubating an extract for both 6 hours and 16 hours. This option may be selected initially, or it may be used in follow-up testing with sample extracts that produced high responses (high contamination) in the 16-hour test. Since PAHs can be degraded by the enzymes in the cells, they will produce a maximal response at 6 hours, and then the response at 16 hours of exposure will decrease by about a factor of 5. However, the test response to the chlorinated compounds is slower, where the ratio of the 16-hour response to that observed at 6 hours is often 2 to 4. The response to PAHs will always decrease from 6 hours to 16 hours and the level of response to chlorinated hydrocarbons will always increase. Mixtures of PAHs and chlorinated compounds will produce a ratio between 0.2 to 1, when the 16-hour response is divided by the 6-hour response. The ratio will depend upon the specific compounds present in the mixture and their potencies. By comparing responses at two time intervals, it is possible to forecast whether the sample contains only PAHs, only chlorinated species, or a mixture of these chemicals.

Based on the degree of change observed between the two intervals, it is possible to predict whether the response is being governed by PAHs with only slight chlorinated effects, or whether the response is mostly due to chlorinated species with small PAH effects (see Section 12.7). This information can be useful in identifying hot spots for further investigation, or in identifying a subset of samples for additional chemical analysis.

11.7.1 If the optional 6-hour and 16-hour testing is to be conducted, prepare a duplicate plate containing three replicates of each sample extract, plus replicate standards and blanks, using the same procedure described in Secs.11.6.1 to 11.6.6. One plate will be incubated for 6 hours and the second for 16 hours.

11.7.2 Process one plate after 6 hours and the second plate after 16 hours, using the procedures described in Secs. 11.6.7 to 11.6.15.

11.7.3 Calculate the ratio of the 16-hour to 6-hour responses as described in Sec. 12.4.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Calculate the mean blank response, the fold induction, the mean fold induction, the standard deviation, and the relative standard deviation for the three replicates of each standard and sample, and using the formula below.

$$\text{mean blank response} = \frac{\sum_{i=1}^{n} (\text{RLU of blank})_i}{n}$$

$$\text{fold induction} = \frac{\text{RLU of each sample replicate}}{\text{mean blank response}}$$

$$\text{mean fold induction} = \overline{\text{fold induction}} = \frac{\sum_{i=1}^{n} (\text{fold induction})_i}{n}$$

$$SD = \sqrt{\frac{\sum_{i=1}^{n} \left([\text{fold induction}]_i - \overline{\text{fold induction}}\right)^2}{n-1}}$$

$$RSD = \frac{SD}{\text{mean fold induction}} \times 100$$

where n is the number of replicates analyzed, i.e., 3.

12.2 If the mean fold induction for a sample extract is over 100, then the sample extract should be diluted and retested. If the RSD of the three replicates is greater than 20%, then the sample extract should be retested until the RSD is less than 20%.

12.3 If, as usual, the volume of the sample extracts applied to the cells in the test was the same for all samples, then the relative concentrations of the inducing compounds in the group of samples can be compared. If the volume of the samples applied to the test were not the same, care must be taken to use the proper multiplication factor to obtain the total fold induction per 1-mL volume of extract (see Sec. 12.6.3).

12.4 If both 16-hour and 6-hour testing were conducted, the ratio of the 16-hour to 6-hour responses for each sample should be calculated as follows.

$$\text{Response Ratio} = \frac{\text{mean fold induction at 16 hours}}{\text{mean fold induction at 6 hours}}$$

12.5 At a minimum, the results from screening samples may be reported as the mean fold induction of the sample, plus the mean fold induction of the standard run with the sample and the concentration of the specific analyte used for the calibration. These data will allow the screening results to be used in a range finding mode, e.g., to determine if the sample response is above or below the response of the specific calibration standard. Additional data reporting options are described in Sec. 12.6.

12.6 Converting test results to TCDD toxic equivalents or benzo[a]pyrene equivalents

12.6.1 Depending upon the needs of the project, it may be appropriate to express the data in terms that relate to key environmental contaminants. Based on published information and standard curves, it is possible to convert the test data to an equivalent concentration of 2,3,7,8-TCDD (e.g., the TEQ) or an equivalent concentration of benzo[a]pyrene (e.g., B[a]P equivalents).

12.6.2 Since this test detects both PAHs and chlorinated hydrocarbons (coplanar PCBs, dioxins, furans) it may be appropriate to express the data in terms of both B[a]P Equivalents and a TEQ. Interpretation of the relative magnitude of these two classes of contaminants will be enhanced by conducting the two-time-interval test as discussed in Sec. 11.7 (also see Figure 4).

12.6.3 In order to express the results in terms of either the TEQ or the B[a]P equivalents, determine the total amount of inducing compounds in the entire extract. This may be accomplished by multiplying the mean fold induction from actual aliquot of the extract that was tested by the inverse of the proportion of the total sample extract that was tested. For example, if the sample aliquot tested is 10 µL and the total volume of the concentrated extract is 1 mL, then multiply the test results by 100 (i.e., 1 mL/10 µL) to obtain the total in the 1 mL extract.

This total amount can be converted to the concentration in the original sample by dividing by the initial sample volume or weight. For a 1-L water sample, divide the total amount by 1 L. For a solid sample, divide the total amount by the dry weight of the sample in grams to determine fold induction per dry gram. This value may also be expressed in terms of the weight in kilograms by multiplying the concentration by 1000.

12.6.4 A standard curve for a PCDD/PCDF mixture has demonstrated that fold induction per g is equal to the dioxin toxic equivalents (TEQ) in pg/g dry weight. Additional testing has shown that fold induction per g may be converted to benzo[a]pyrene equivalents (µg B[a]P EQ/g dry weight), by dividing by 60 (response to 1 µg of B[a]P).

12.7 When the optional 6-h and 16-h tests have been conducted, the difference between the two responses should be evaluated. When only PAHs are present, the test response will *decrease* between 6 and 16 hours by about a factor of 5, and the response ratio

calculated in Sec.12.4 will be approximately 0.2. When only PCDDs/PCDFs/PCBs are present (e.g., no PAHs present), the test response will *increase* between 6 and 16 hours and the response ratio calculated in Sec. 12.4 will be greater than 1, and often much greater than 1.

When both PAHs and the chlorinated target compounds are present, the response ratio will fall between the two extremes. A value between 0.2 and 1 indicates that both PAHs and the chlorinated target compounds are present. Figure 4 includes examples of the test responses when such mixtures are present.

13.0 METHOD PERFORMANCE

13.1 Table 1 illustrates the sensitivity of the RGS assay to a variety of environmental contaminants. The detection limit estimates in this table are the concentrations necessary to produce a response at least 10 times the background response. The data in the column for the final extract (in the 2-mL well) indicate the relative sensitivity of the assay to the three classes of compounds, with the greatest sensitivity for the dioxins and furans, followed by the PCBs, and then the PAHs.

13.2 Table 2 contains a list of those environmental contaminants that have been tested by the developer and did not induce a response in the RGS assay. The compounds include a variety of organochlorine pesticides, non-coplanar PCBs, and low molecular weight PAHs.

13.3 Figure 5 illustrates the response of the RGS assay to low concentrations of 2,3,7,8-TCDD, 2,3,7,8-TCDF, OCDD, and OCDF. Almost no response is observed from OCDD, and it would take 20,000 times the OCDF concentration to produce the same response as that of 2,3,7,8-TCDD. In comparison, the TEF for OCDF is 0.001, indicating that OCDF is at least 1,000 times less toxic than 2,3,7,8-TCDD.

13.4 Figure 6 shows the test responses to both a standard containing a mixture of dioxins/furans and a standard containing a mixture of PAHs. The mixture of PAHs, which also contained 2- and 3-ring aromatics not identified by this method, produced a strong response (10 times background) in the test system at a concentration of 75 ng/mL, which is equivalent to a soil sample concentration of about 750 ug/kg. The test is very sensitive to benzo(k)fluoranthene, and quite sensitive to six other high molecular weight PAHs.

13.5 The performance of the P450 RGS test during a NOAA investigation of Sabine Lake, Texas is illustrated in Figure 7. There was a strong positive correlation ($r^2 = 0.84$) between the RGS test results, expressed as B[a]P equivalents, and the total PAH results determined by a GC/MS method. The B[a]P values were 10 to 20 times higher than total PAHs. This is likely because there are many unidentified hydrocarbons in sediment extracts that could induce this system, and there is strong induction by only ng/g concentrations of some compounds (e.g. benzo[k]fluoranthrene). The difference in magnitude between the RGS results and the GC/MS was relatively consistent across all 65 samples from this project and the samples with the highest RGS results corresponded to those with the highest GC/MS results. When the samples were ranked by the concentrations determined by both techniques, there were no samples that represented false negative results for the RGS technique.

13.6 Figure 8 illustrates the performance of the RGS method in the detection of mixtures of PCDDs/PCDFs in soil samples from a dioxin cleanup site in the Pacific Northwest. A total of 24 samples were collected at this site and tested with the RGS procedure. Six of those 24 samples representing a range of RGS responses were also analyzed for PCDDs/PCDFs using Method 8280, a GC/MS procedure. The results of the GC/MS analyses, expressed as TEQ, are plotted against the TEQ estimated from the RGS procedure. There was a good positive correlation between the two techniques (r^2 = 0.89), and the RGS values were about a factor of 2 higher than the GC/MS values. The samples with the highest RGS responses also had the highest results using Method 8280 and there was no evidence of false negative results for the RGS procedure.

13.7 Figures 9 and 10 illustrate the results for soil samples containing PCBs. The results from this method are compared to instrumental analyses (Method 8082) performed for 32 individual PCB congeners, including all the coplanar PCBs. The correlations between the RGS results and the GC/EC result were strong for both total coplanar PCBs and the total of the PCB congeners measured (r^2 = 0.79 - 0.83).

13.8 Marine mussels (*Mytilus edulis*) were deployed at various sites in San Diego Bay in order to determine the bioaccumulation of various toxicants over a one-month period. Figure 11 shows the correlation of the RGS test results with GC/MS analyses of the PAHs in these tissues. The RGS results are approximately 10 times higher than the chemical analyses, but the GC/MS method did not include all possible PAHs. In addition, the RGS induction is greater for some compounds than others. The RGS results and the GC/MS results showed a positive correlation with r^2 = 0.82 and no evidence of false negative results.

13.9 Figure 12 shows the RGS assay responses to TCDD added directly to the cells, and to extracts of water samples spiked with TCDD. Both curves demonstrate a strong concentration-response relationship and good linearity over the range tested, but the water extracts produced somewhat lower induction, possibly due to losses during extraction of the water samples with methylene chloride and the concentration of the extract.

13.10 Figure 13 shows the 6-hour and 16-hour responses of 17 soil samples that contained PCDDs/PCDFs. Note that the responses at 16 hours were much stronger than the responses at 6 hours. However, most of these extracts produced a 16-hour response greater than 100 fold induction. As noted earlier, sample extracts that exhibit a response of greater than 100-fold should be diluted and reanalyzed.

Figure 14 illustrates the effects of diluting the extracts of the same 17 samples by a factor of 10. Comparing the results of the original extracts to those diluted extracts allows the user to distinguish those samples that also have a substantial contribution from PAHs that is not evident in the original extracts which produced an induction of over 100-fold. Of the 17 samples shown in Figures 13 and 14, only three samples (6, 7, and 8) are predominated by PCDDs/PCDFs, while the other 14 samples exhibit the moderate to significant decreases in the response at 16 hours that are indicative of the presence of PAHs.

13.11 Table 3 and Figure 15 illustrate the relationship between the RGS values reported on blind samples and Method 8290 data for the same samples. The samples represent four different projects, ranging from New York Harbor sediments to soils from a wood treating site.

The TEQ values estimated from the RGS responses are between 1.8 and 200 times higher than the TEQ values calculated from the high resolution GC/MS data, indicating that the RGS assay is responding to some components of the extract that are not measured by the instrumental analyses or that are not included in the TEQ calculation from those analyses. Regardless, the correlations between GC/MS TEQs and RGS TEQs ranged from 0.69 to 0.99, demonstrating that the relative magnitudes of the RGS results can be used to rank samples by concentration. The data in Figure 15 are plotted on a log-log plot in order to fit them all in the same figure and still be readable. The correlations in Table 3 were calculated from the original results.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of a waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasiblely reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society.

15.0 WASTE MANAGEMENT

Laboratory waste management procedures must be consistent with federal, state and local regulations. See Sec. 7 for a list of reagents used in this procedure. The laboratory should make an effort to collect and recycle its waste solvents whenever possible.

16.0 REFERENCES

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17.0 TABLES AND FIGURES

TABLE 1

P450 RGS ASSAY DETECTION LIMIT ESTIMATES

Analyte	Concentration Producing a Response of 10 times Background		
	In Final Extract (ng/mL)	In 1-L Water Samples (µg/L)	In 40-g Soil Samples (µg/kg)
Dioxins and Furans			
2,3,7,8-TCDD	0.003	0.0003	0.008
2,3,7,8-TCDF	0.1	0.01	0.25
Dioxin/Furan Mixture	0.07	0.007	0.17
OCDF	50	5	125
OCDD	2500	250	6250
Polychlorinated Biphenyls			
PCB # 81	0.5	0.05	1.25
PCB #126	4.0	0.4	10
PCB #77	500	50	1250
PCB #114	300	30	750
PCB #118	2500	250	6250
PCB # 123	2500	250	6250
PCB #169	15000	1500	37500
PAHs			
Benzo[k]fluoranthene	6	0.6	15
Dibenzo[a,h]anthracene	100	10	250
Benzo[b]fluoranthene	200	20	500
Indeno[1,2,3-cd]pyrene	100	10	250
PAH Mixture	100	10	250
Benzo[a]pyrene	250	25	625
Benzo[a]anthracene	600	60	1500
Benzo[a]fluorene	1000	100	2500
Chrysene	600	60	625
Benzo[g,h,i]perylene	20000	2000	50000

These estimates are based on single laboratory testing and are provided for illustrative purposes only.

TABLE 2

COMPOUNDS TESTED WHICH DO NOT INDUCE A RESPONSE IN THE P450 RGS ASSAY

Pesticides	Low Molecular Weight PAHs	PCBs
Aldrin	Acenaphthene	Non-coplanar PCBs
Chlordane	Anthracene	Hexabromobiphenyl (surrogate)
Dieldrin	2-Methylanthracene	
p,p′-DDT	9-Methylanthracene	
o,p′-DDT	9,10-Dimethylanthracene	
p,p′-DDE	Fluorene	
o,p-DDE	1-Methylfluorene	
p,p′-DDD	Naphthalene	
o,p-DDD	2,6-Dimethylnaphthalene	
Endosulfan II	2,3,5-Trimethylnaphthalene	
Endosulfan sulfate	Phenanthrene	
Endrin	2-Methylphenanthrene	
Endrin aldehyde	3,6-Dimethylphenanthrene	
Heptachlor		
Heptachlor epoxide		
α-BHC		
β-BHC		
γ-BHC (Lindane)		
δ-BHC		
Pentachlorophenol		
Toxaphene		

TABLE 3

COMPARISON OF RGS TEQ VALUES WITH GC/MS RESULTS FOR METHOD 8290

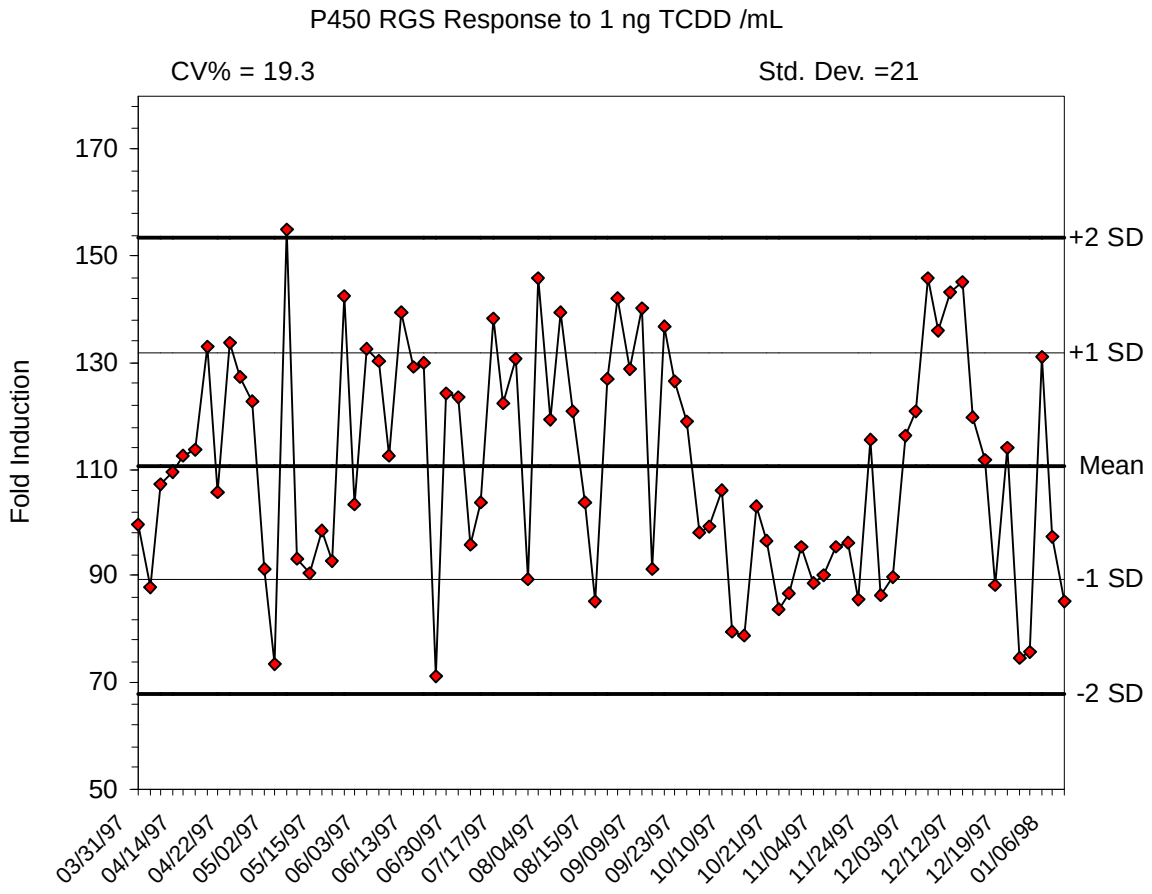
Sample Type	No. Samples	RGS-to-GC/MS Ratio	Correlation (r^2)
Island Soils - A	20	3.3	0.99
Island Soils - B	49	21	0.97
NY Harbor Sediments	22	200	0.81
Sediments from Florida	27	6	0.69
Soils from Wood Treating Site	64	1.8	0.79

The RGS-to-GC/MS ratio is the ratio of the TEQ values determined by each technique.

The correlation coefficients were calculated for the data from each sample type.

FIGURE 1

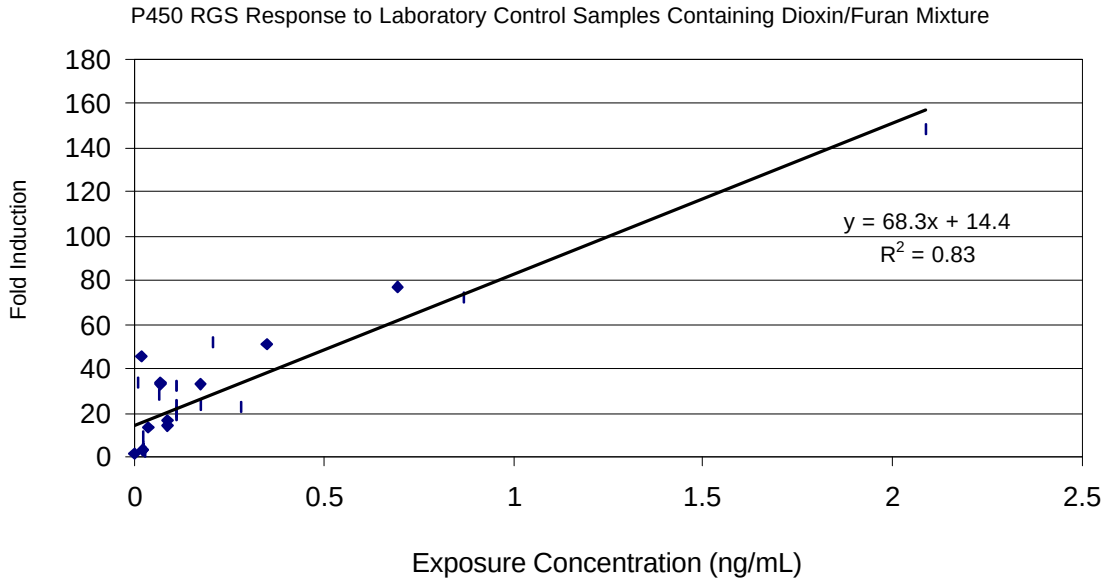
EXAMPLE LABORATORY QUALITY CONTROL CHART



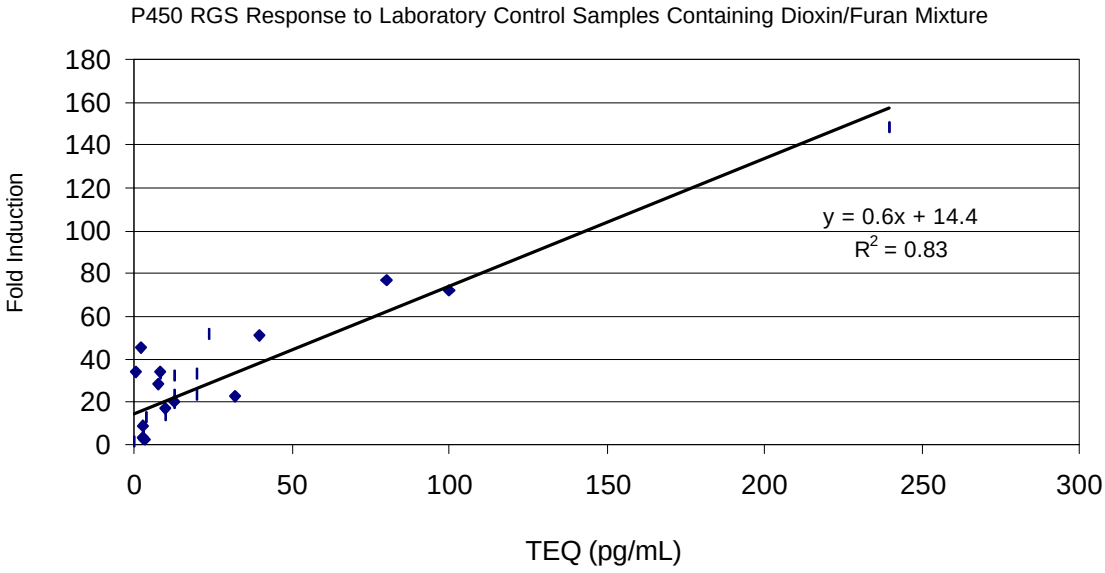
These data are provided for illustrative purposes only. Each laboratory employing this method is responsible for tracking long-term performance of the assay in some fashion.

FIGURE 2

EXAMPLE CONCENTRATION-RESPONSE CURVES FOR A PCDD/PCDF MIXTURE



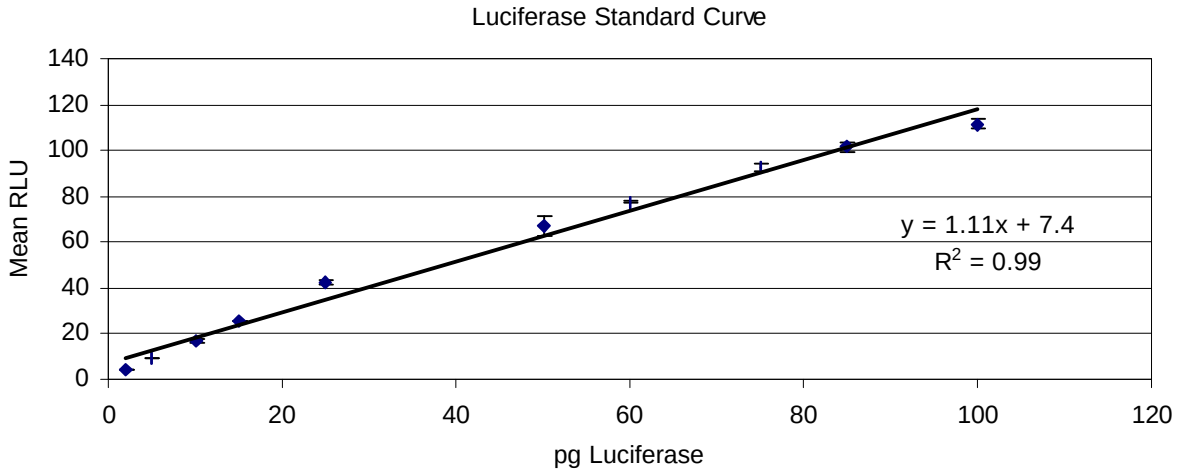
Fold induction results plotted against the total concentration of PCDDs/PCDFs in the mixture. These data are for illustrative purposes only.



Fold induction results plotted against the toxic equivalent concentration (TEQ) of PCDDs/PCDFs in the mixture. These data are for illustrative purposes only.

FIGURE 3

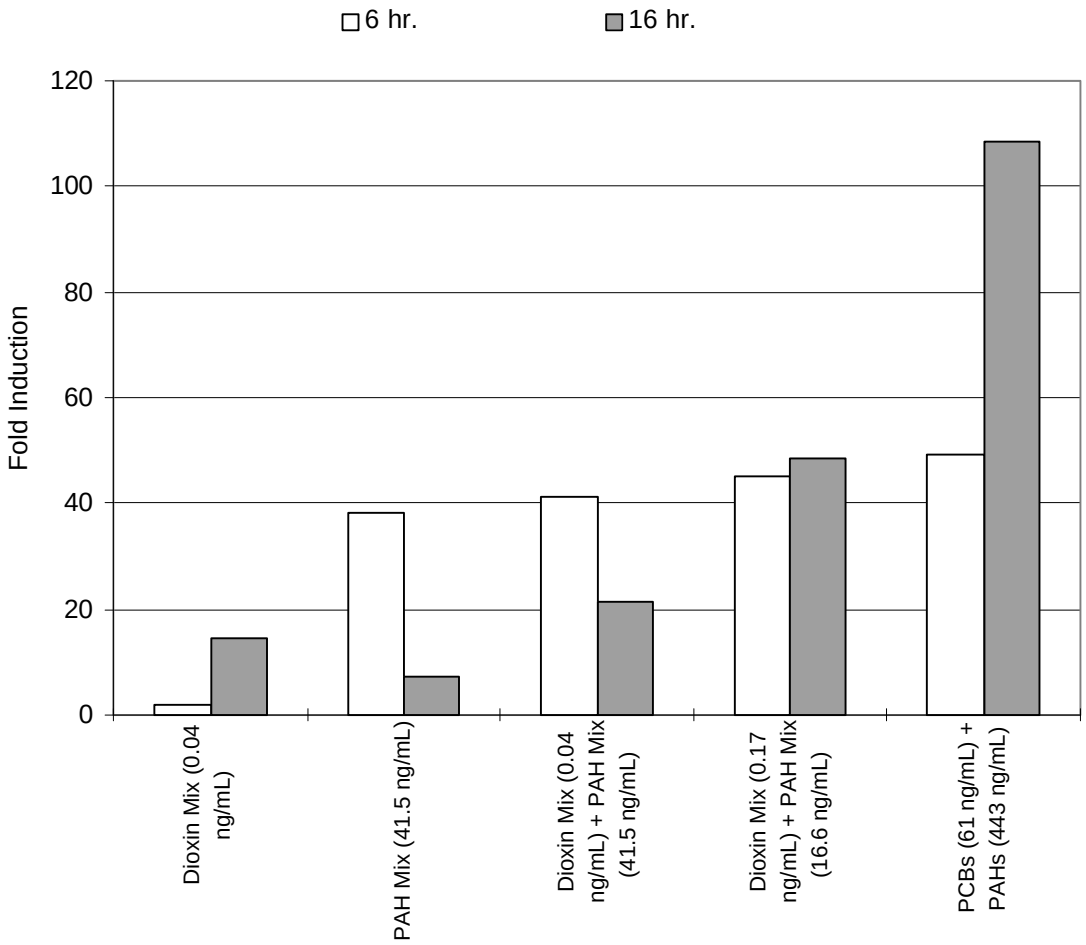
EXAMPLE STANDARD CURVE FOR THE LUMINOMETER



These data are provided for illustrative purposes only.

FIGURE 4

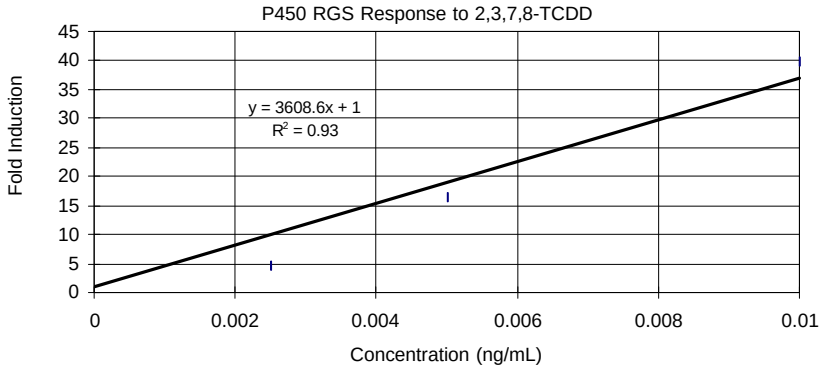
EXAMPLES OF 6-HOUR AND 16-HOUR RESPONSES TO PCDDs/PCDFs, PCBs, AND PAHs

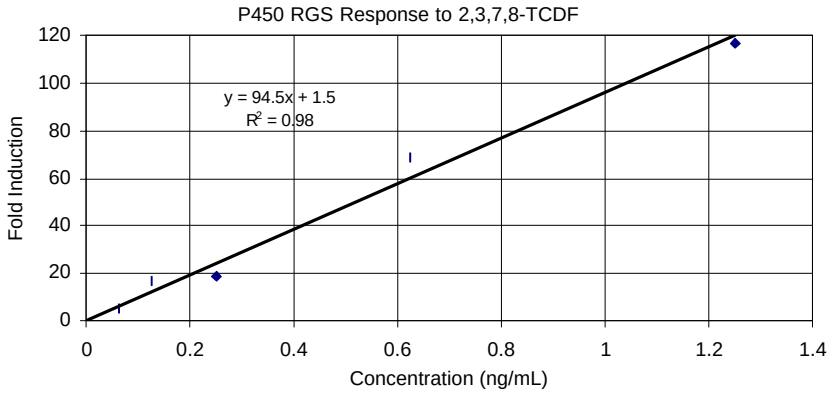


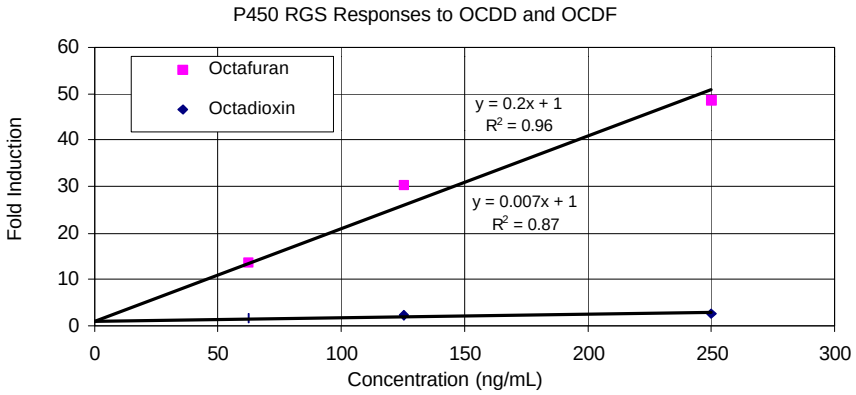
See Sec. 12.7 for additional explanation of the differentiation of the responses due to PCDDs/PCDFs, PCBs, and PAHs.

FIGURE 5

EXAMPLE RESPONSES OF THE RGS ASSAY TO LOW CONCENTRATION STANDARDS CONTAINING 2,3,7,8-TCDD, 2,3,7,8-TCDF, OCDD, AND OCDF



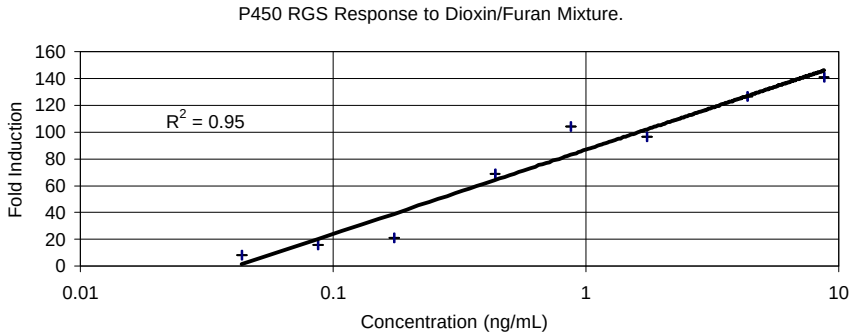


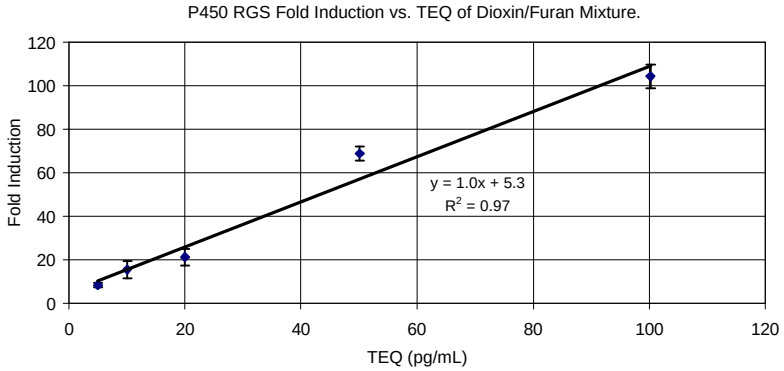


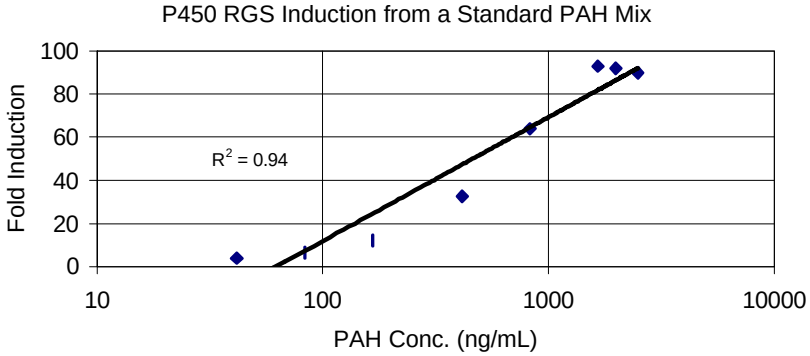
Pre-release version - This method has NOT been released by OSW as part of Update IV

FIGURE 6

EXAMPLE RESPONSES OF THE RGS ASSAY TO LOW CONCENTRATION STANDARDS CONTAINING A PCDD/PCDF MIXTURE AND A PAH MIXTURE



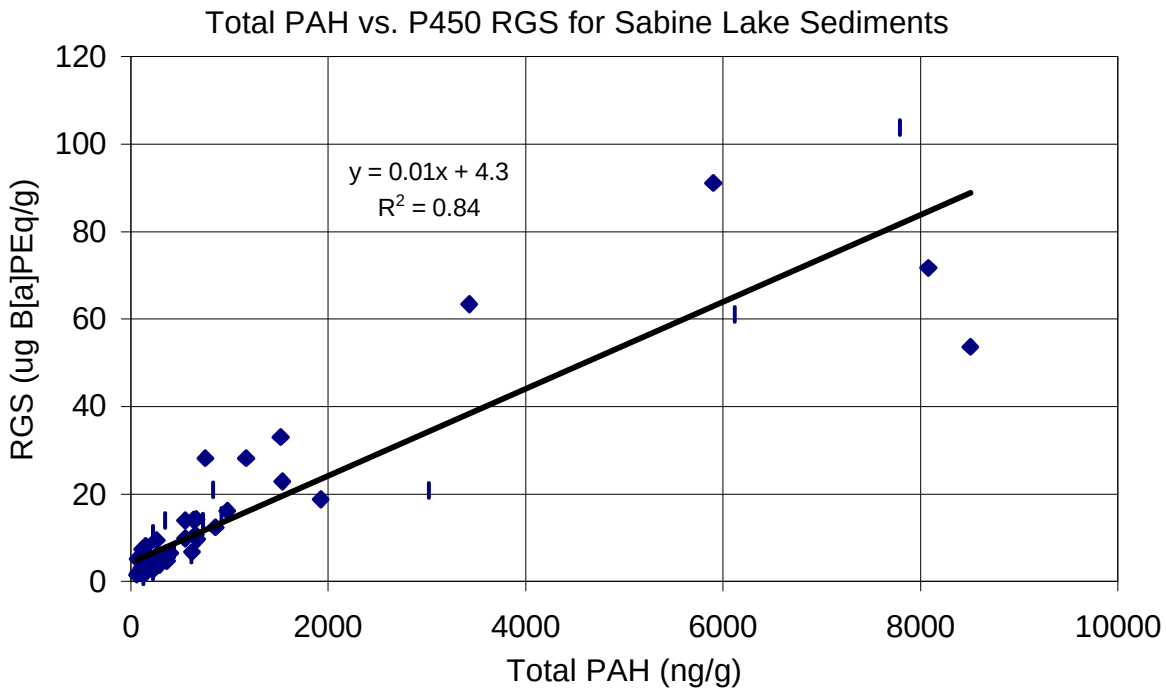




Note the change in horizontal scales. The upper PCDD/PCDF and PAH figures use a log scale, while the TEQ figure in the center uses an arithmetic scale.

FIGURE 7

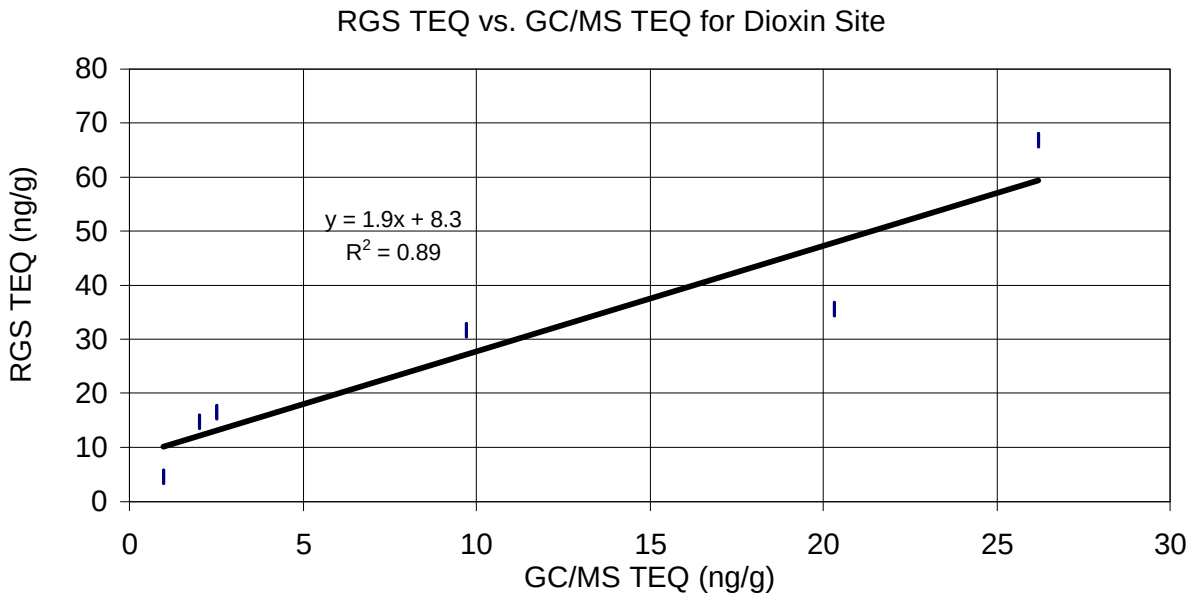
RGS RESULTS FOR PAHs PLOTTED AGAINST GC/MS RESULTS FOR TOTAL PAHs FROM A NOAA STUDY OF SEDIMENTS FROM SABINE LAKE, TEXAS



Pre-release version - This method has NOT been released by OSW as part of Update IV

FIGURE 8

PERFORMANCE OF THE RGS METHOD IN THE DETECTION OF MIXTURES OF PCDDs/PCDFs IN SOIL SAMPLES FROM A DIOXIN CLEANUP SITE



A total of 24 samples were collected at this site and tested with the RGS procedure. Six of those 24 samples, representing a range of RGS responses, were also analyzed for PCDDs and PCDFs using Method 8280, a GC/MS procedure. The results of the GC/MS analyses, expressed as the TEQ, are plotted against the TEQ estimated from the RGS procedure.

FIGURE 9
RGS RESULTS FOR PCBs PLOTTED AGAINST GC/EC RESULTS
FOR TOTAL PCB CONGENERS

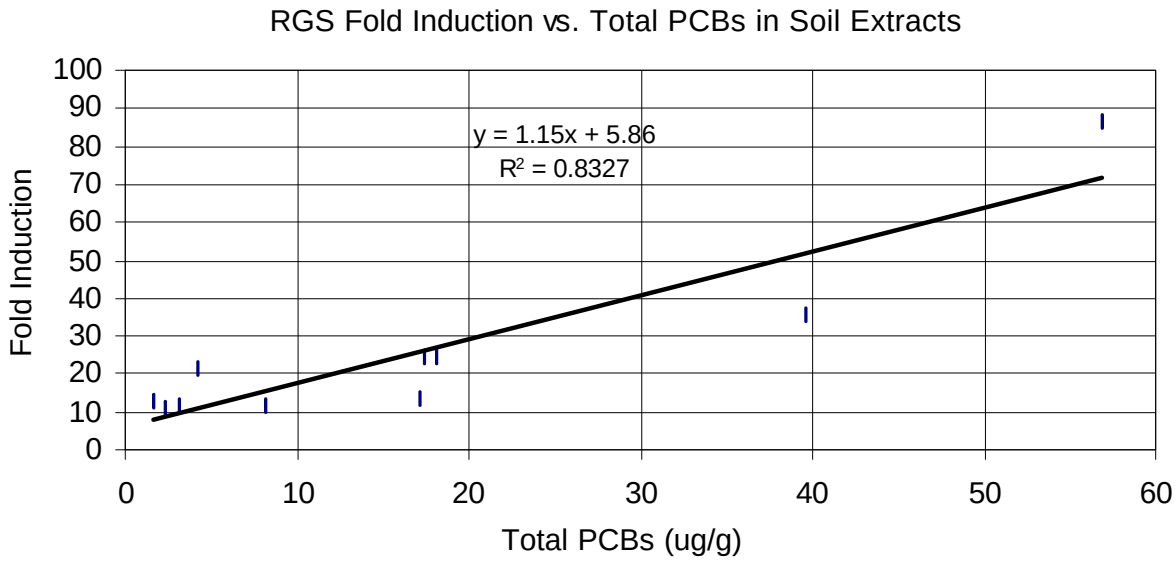
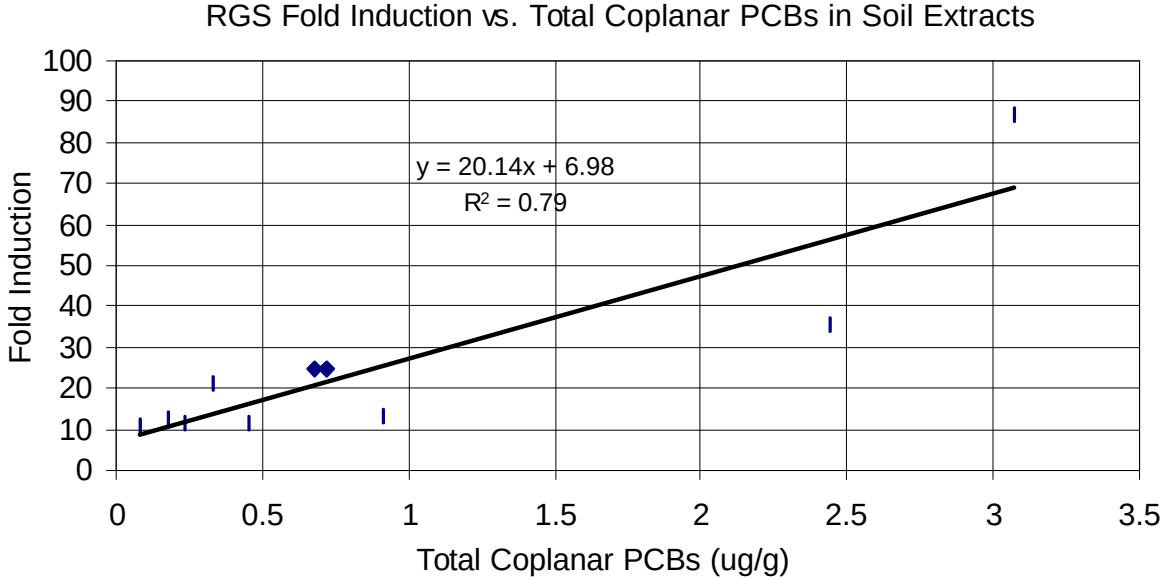


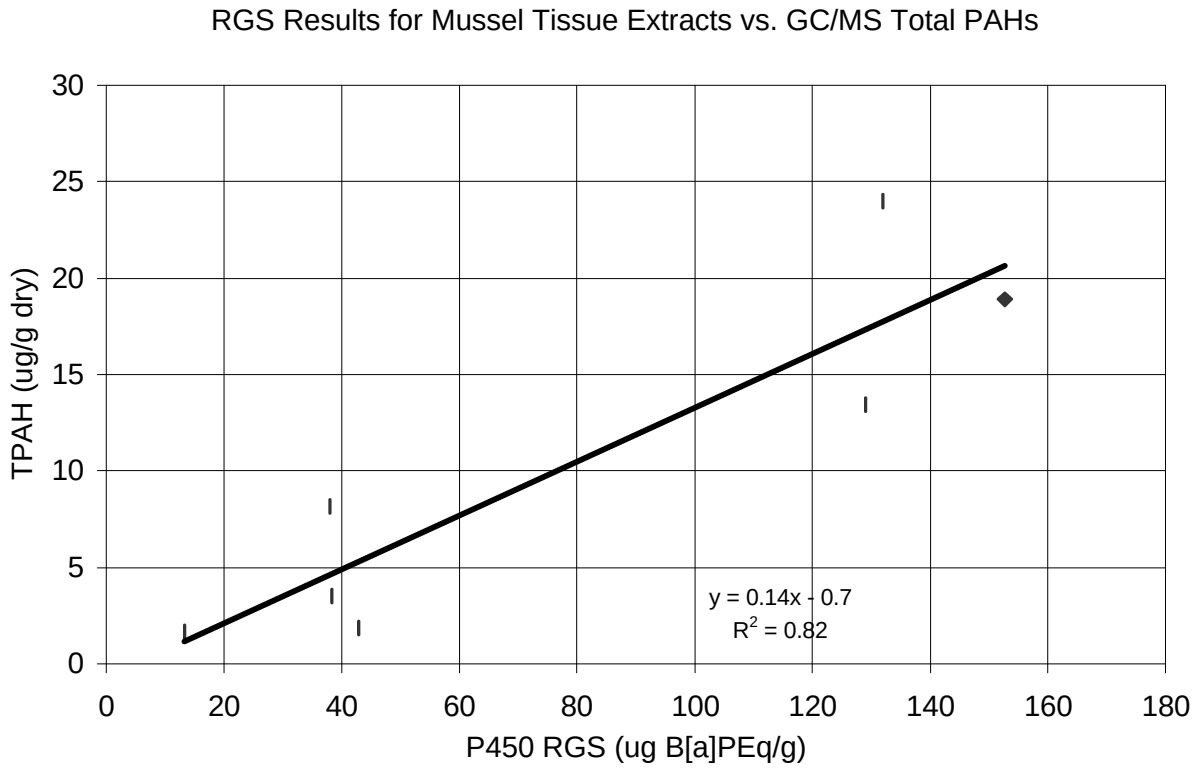
FIGURE 10
RGS RESULTS FOR PCBs PLOTTED AGAINST GC/EC RESULTS
FOR TOTAL COPLANAR PCBs



Pre-release version - This method has NOT been released by OSW as part of Update IV

FIGURE 11

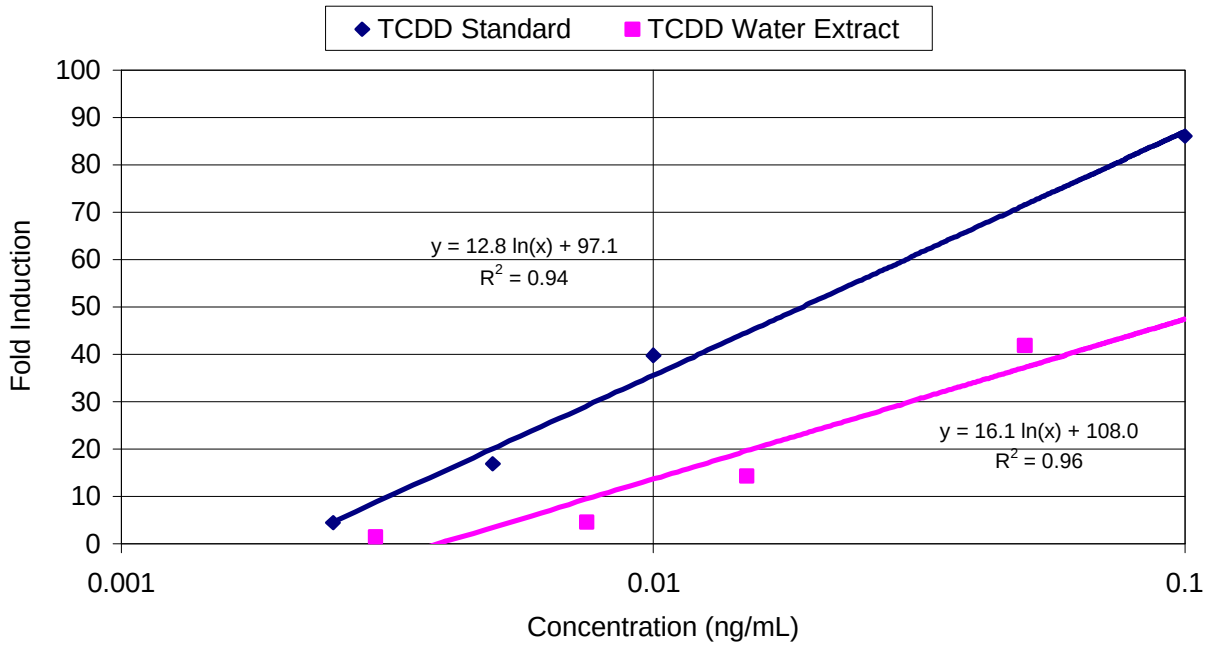
RGS RESPONSES AND GC/MS RESULTS FOR TOTAL PAHs IN MUSSEL TISSUE EXTRACTS



RGS responses are expressed as B[a]pyrene equivalents. See Sec. 12.6.

FIGURE 12

COMPARISON OF RGS RESPONSES TO STANDARDS CONTAINING 2,3,7,8-TCDD AND EXTRACTS OF WATER SAMPLES SPIKED WITH 2,3,7,8,-TCDD



Note that this figure uses a log scale for the x-axis.

FIGURE 13

RGS RESPONSES TO SOIL EXTRACTS INCUBATED FOR 6 HOURS AND 16 HOURS

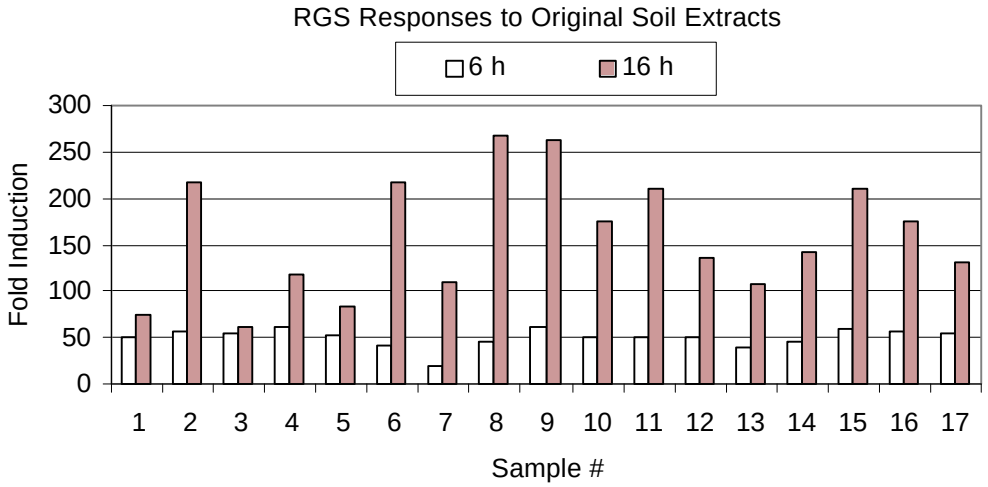
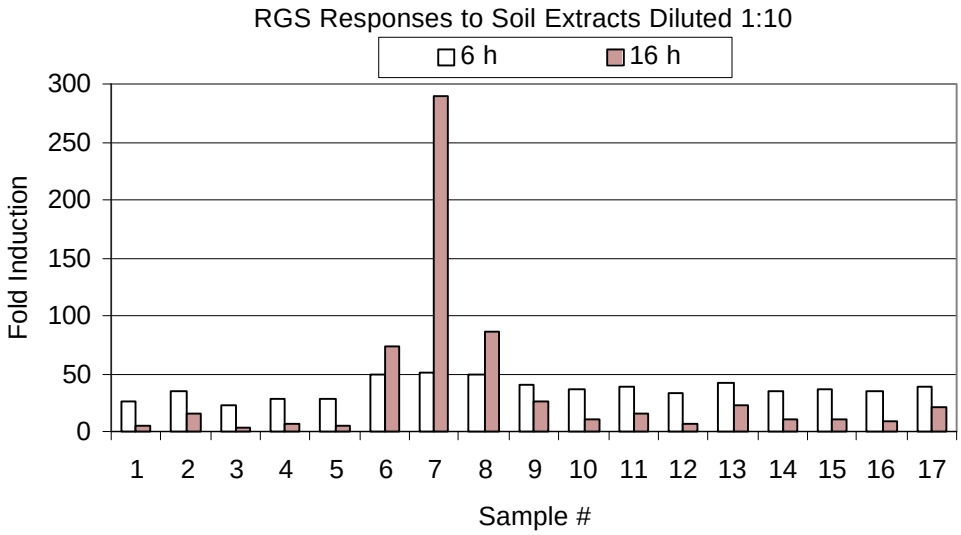


FIGURE 14

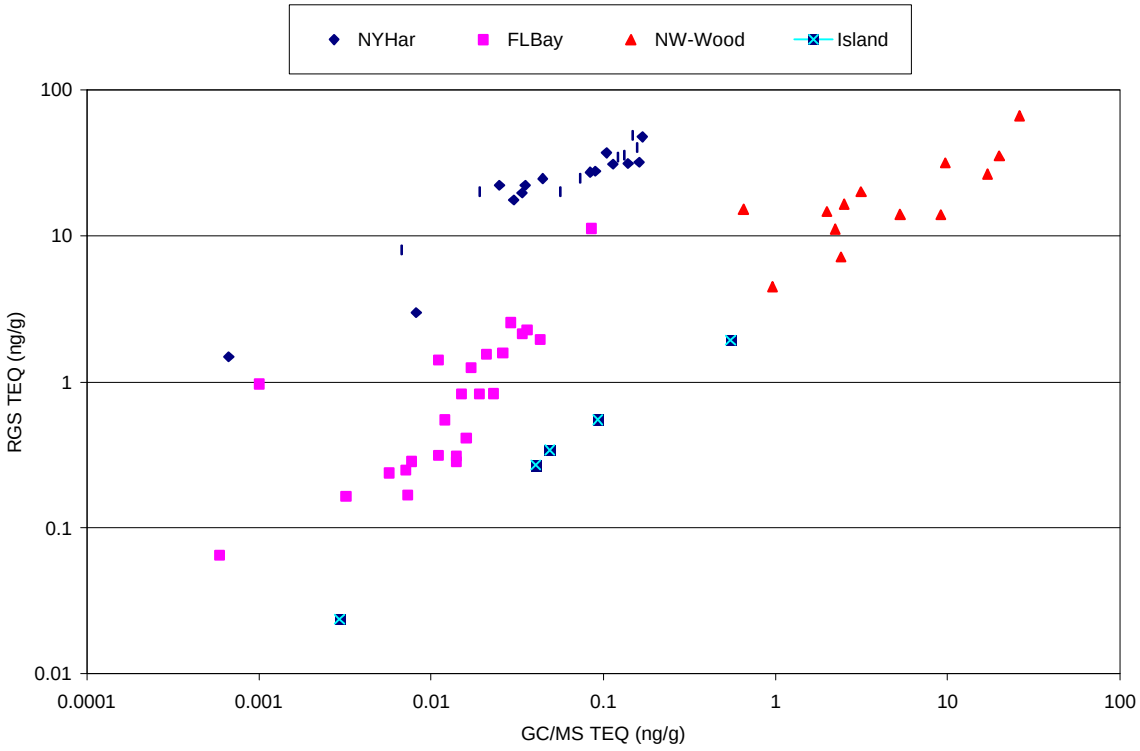
RGS RESPONSES TO SOIL EXTRACTS INCUBATED FOR 6 HOURS AND 16 HOURS AND DILUTED 1:10



Note that the response at 6 hours in the diluted extracts in Figure 14 is generally higher than the response at 16 hours in those diluted extracts. The 6-hour response is characteristic of the presence of PAHs, but in the original extracts in Figure 13, it is overwhelmed by the dioxin responses that are well over 100-fold.

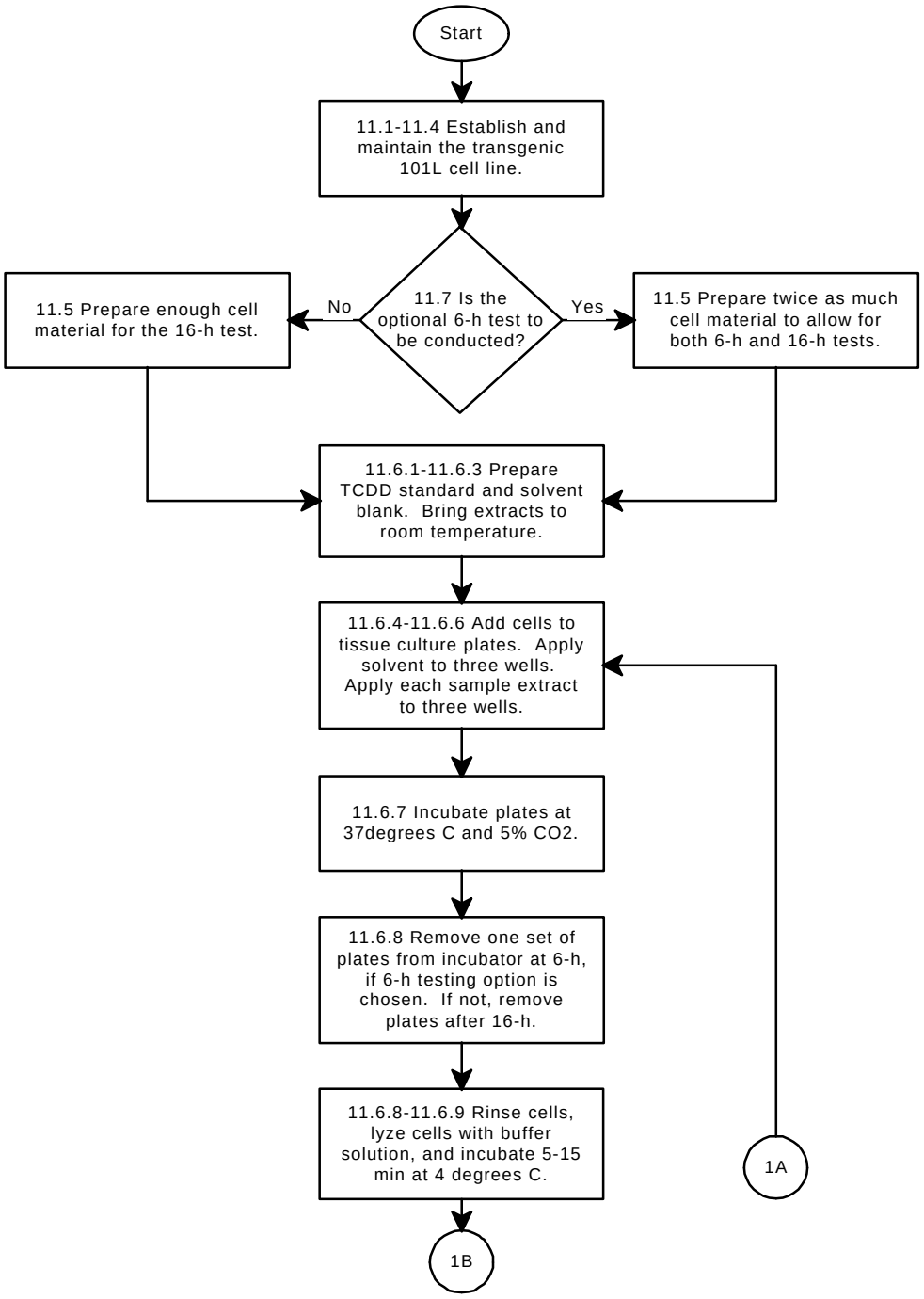
FIGURE 15

COMPARISON OF THE RGS RESPONSES AND THE GC/MS RESULTS FOR SAMPLES FROM FOUR DIFFERENT TYPES OF DIOXIN-CONTAMINATED SITES

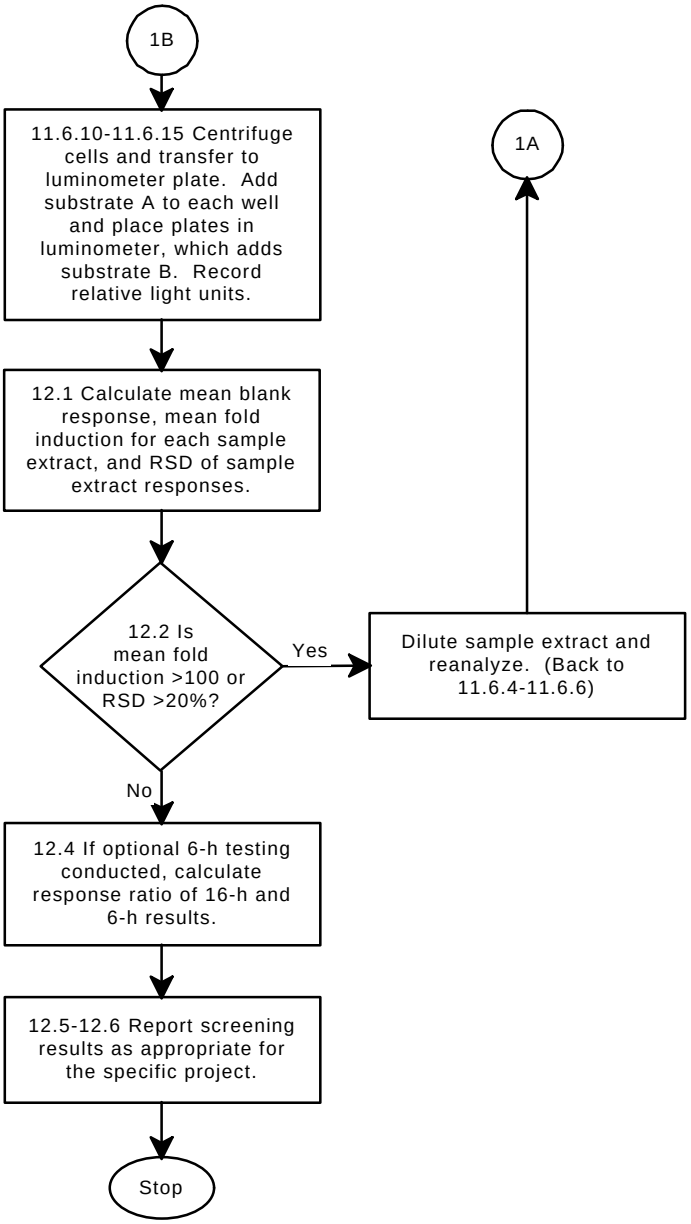


Note that this figure uses a log-log scale.

SCREENING EXTRACTS OF ENVIRONMENTAL SAMPLES FOR PLANAR ORGANIC COMPOUNDS (PAHs, PCBs, PCDDs/PCDFs) BY A REPORTER GENE ON A HUMAN CELL LINE



Pre-release version - This method has NOT been released by OSW as part of Update IV



B[a]PEq - Benzo[a]pyrene Equivalents. Used to express potency of a test substance by its RGS fold induction in relation to the potency of benzo[a]pyrene (B[a]P). As such, B[a]PEq is a concept that is defined specific to this method.

CYP1A1 - Cytochrome P450 1A1. The major gene subfamily of cytochrome P450 in humans mediated by the Ah receptor.

Fold induction - The response of an enzyme divided by response of a control or solvent blank.

Geneticin (or G418) - An antibiotic used as a selection agent, toxic to bacteria, yeast, protozoa, helminths, and mammalian cells so that resistance is conferred by a gene of bacterial origin (e.g. neomycin).

Inducing compounds - Compounds which induce the transcription of the CYP1A1 gene (includes many PAHs, coplanar PCBs, PCDDs/PCDFs).

Luciferase - An enzyme found in the firefly that catalyzes the conversion of luciferin to oxyluciferin, giving off CO_2 and light.

Luciferin - The substrate for the luciferase reaction that produces light.

Plasmid - A circular molecule of double-stranded DNA carrying genes. Used in transfection.

Reporter gene - A gene used in transcriptional studies whose expression is readily measured (e.g. luminescent) or exogenous to the species of study.

RGS - Reporter Gene System. An *in vitro* assay using the luciferase reporter gene in a human hepatoma cell to study induction of the CYP1A1 gene.

TEF - Toxic Equivalency Factor. Used to describe the potency of an individual compound in relation to 2,3,7,8-TCDD. TEF values for the PCDDs and PCDFs have been established by international consensus (see Reference 8).

TEQ - Toxic Equivalent. A concept used to express the potency of a substance in relation to the potency of 2,3,7,8-TCDD. The TEQ is the sum of the products of the concentrations of the individual compounds times their respective TEF values (see Reference 8).